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IMMUNE REACTIONS WITH DIPLOCOCCI ISOLATED FROM MEASLES AND RUBELLA

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Tunnicliff[1] has described gram-positive cocci which were isolated in anaerobic cultures from the blood of measles and rubella patients. The measles coccus is small and round, the rubella coccus is larger, elongated, with pointed ends and a capsule. Both generally occur in pairs. Diplococci similar to those isolated from the blood were also cultivated from the throat, nose, eye and ear of measles patients, and from the throat of rubella patients. Immune reactions in patients with measles and rubella were studied and specific opsonins for the measles and rubella diplococci were found to develop during the course of measles and rubella, respectively, disappearing with recovery. There were no changes in the opsonic content of the blood in either disease for the Streptococcus viridans, pneumococcus or the poliomyelitis coccus in any of the serums examined. Agglutination experiments were unsatisfactory on account of the tendency of the cocci to agglutinate spontaneously.

COMPLEMENT FIXATION

A few complement fixation tests have been made with measles and rubella serum using as a rule a suspension of the diplococci isolated from the blood of measles patients, but also a mixture of strains, as the measles antigen, and a mixture of strains isolated from the blood and throat of rubella patients for the rubella antigen.

The cocci for the fixation tests were grown for 24 hours in ascitic dextrose broth (0.2% dextrose, 0.6% acid to phenolphthalein, 10% ascitic fluid), removed by centrifugation, washed and suspended in sterile normal salt solution. The mixtures were then heated at 56 C. for 30 minutes.

The antisheep-rabbit system was used in one-tenth the volume of the original Wassermann test. The anticomplementary unit of antigen was determined, and the hemolytic system was standardized by an amboceptor titration. In the test, the serum remained constant at 0.02 c c; the antigen varied from ¼-¹⁄₁₂₀ of the anticomplementary unit. Fresh guinea-pig serum, 0.1 c c of a 1:10 dilution (2 units), was used as a complement. Serum, antigen, and complement were incubated at 37 C. for 1 hour. Two units of previously titrated antisheep amboceptor and 0.1 c c of sheep corpuscles in 5% suspension were added to each tube, and the whole incubated for 30 minutes. The customary controls (antigen, serum and hemolytic system) were set up with each test.

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[1] Jour. Am. Med. Assn., 1917, 68, p. 1028; Jour. Infect. Dis., 1918, 22, p. 462.

Fifteen cases of measles were examined, 8 before the 8th day; the others during the 2nd and 3rd weeks following the infection. The 8 early cases showed some fixation on 1 day, but were examined too infrequently to determine the duration. Five patients were examined twice and not on successive days, 1 was examined only once, and 1 every 3rd day for 12 days. One fixed with the homologus blood coccus, 6 with an heterologous blood coccus, 3 of these fixed also with the mixed measles antigen, and 1 with the mixed antigen only. The fixation was strong in 1 case only when inhibition occurred in 1/32 of the anticomplementary unit, in 4 inhibition occurred in 1/16 of the anticomplementary unit, and 3 in 1/4 of the anticomplementary unit. The serum of 3 patients also caused inhibition in 1/4 of the anticomplementary unit, of the rubella antigen and a hemolytic streptococcus (scarlet fever gland) antigen.

Of the 13 rubella serums, only 7 were examined more than once, and then only every other day for 3 or 4 days. They were all studied during the week of the attack. Only 5 of the 13 serums showed any fixation for the rubella antigen and then only to a slight degree (1/4 the anticomplementary unit) and none for the measles antigen. Nine serums were tested with hemolytic streptococcus antigen and 4 showed strong fixation (1/132 to 1/64 the anticomplementary unit) and 4 slight fixation. When the patient's serum was examined more than once the positive fixation with the hemolytic streptococcus antigen was found to persist. One of the patients showing strong fixation, had both scarlet fever and rubella, and hemolytic streptococci were present in the throat in some of the other rubella patients examined at the end of the attack.

The results indicate that the serum of measles and rubella patients contain complement fixing bodies for their respective diplococci to a slight degree early in the disease at about the same time the increase in opsonins occurs.

IMMUNE REACTIONS IN RABBITS INJECTED WITH COCCI FROM MEASLES AND RUBELLA

On account of the frequent presence of nonhemolytic diplococci and streptococci in normal and pathologic conditions and the difficulty of differentiating them by their cultural and morphologic characteristics (especially after cultivation), an attempt has been made to differentiate the cocci isolated from measles and rubella from each other and from similar cocci, by immunological methods.

Howell[2] immunized rabbits with some of these measles and rubella diplococci as well as with nonhemolytic and hemolytic streptococci, and concluded that the positive complement fixations could not be grouped in any way that would justify a classification of streptococci based on the complement fixation test.

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[2] Jour. Infect. Dis., 1918, 22, p. 230.

The experiments in this work were therefore confined to agglutination and opsonic determinations.

Rosenow and Gray[3] found agglutination tests useful in differentiating the poliomyelitis streptococcus from streptococci and pneumococci from other sources. Mathers and Tunnicliff[4] found that specific opsonins for the poliomyelitis coccus develop during the course of acute poliomyelitis, and in conjunction with Howell[5] that the serum of rabbits immunized against different strains of poliomyelitis cocci contain opsonins in high concentrations, which were apparently specific for the poliomyelitis cocci. Agglutinins were demonstrable in low dilutions only, and spontaneous agglutination of the cocci interfered with the tests. Nuzum and Willy[6] have found that by means of opsonic determinations of immune horse serum, they were able in 20 instances to identify and separate strains of poliomyelitic cocci from other strains of cocci presenting more or less confusing cultural and morphologic similarities. The point of opsonic extension of the immune horse serum reached as high as 1 : 6,000.

Rabbits, 3-6 months old, were first inoculated intravenously with 10 billion killed diplococci—the measles rabbits with a mixture of 4 strains, 1 blood, 1 eye, 1 ear and 1 throat; the rubella rabbits with a mixture of 2 blood and 2 throat strains. Four days later they were inoculated intravenously with ½ the washings in salt solution of the growth of each organism on 1 blood-agar slant. The dose was increased every 4 days until 7 injections were received, when the dose was reduced to the original dose of living cocci given every 10 days or 2 weeks to maintain the antibody content. The measles cocci, although 3 of the strains were more than a year old, frequently made the rabbits very ill, in which case the dose was diminished and the length of time between injections increased. There was good antibody production after the third injection.

Sixty-five strains of diplococci and streptococci have been examined, 8 from measles, 25 from rubella, and 32 from other sources. The measles and rubella cocci and 28 cocci from other sources were green-producers when first isolated. Four strains were hemolytic streptococci. In the meantime 1 of the measles strains (ear) became more or less hemolytic and 2 of the rubella blood strains lost their power to produce green on blood-agar plates to a large extent, but were still nonhemolytic.

AGGLUTININS

Unheated serum was used in varying dilutions, using the serum of a normal rabbit as control. The experiments were made in capillary pipets in which agglutination is readily observed. The cocci from measles when first isolated agglutinated spontaneously so that no satisfactory tests could be made, but after long isolation they grew diffusely in 1% dextrose and sometimes in calcium carbonate broth and satisfactory agglutination tests could be made. Some of the rubella strains produced good suspensions; the majority were impossible to work with on account of spontaneous clumping. The specimens were incubated at 35 C. for 2 hours and placed in the refrigerator for 20 hours at which time the readings were made.

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[3] Jour. Infect. Dis., 1918, 22, p. 345.
[4] Jour. Am. Med. Assn., 1916, 67, p. 1935.
[5] Jour. Infect. Dis., 1917, 21, p. 292.
[6] Jour. Infect. Dis., 1918, 22, p. 258.

The serum of the immune measles rabbit agglutinated markedly, both the homologous and the heterologous measles strains (Table 1), and also, in low dilutions, 3 rubella strains and 1 coccus from a case of acute bronchitis.

The rubella rabbit serum agglutinated 3 of the homologous strains, the other strain agglutinating spontaneously, but only 1 of the heterologous strains and to a slight extent also a poliomyelitis strain, so that although the majority of the rubella strains agglutinated spontaneously, those that did not do so were generally not agglutinated by the rubella immune rabbit serum.

TABLE 1

AGGLUTINATION TESTS

Sources of Cocci	Dilutions of Serum													
	Serum of Rabbit Immunized With Measles Coccus								Serum of Rabbit Immunized With Rubella Coccus					Normal Rabbit Serum
	1:10	1:20	1:40	1:80	1:160	1:320	1:640	1:1,280	1:10	1:20	1:40	1:80	1:160	1:10
From blood[1] in measles...	++	++	++	++	0				0					0
From blood in measles....	++	++	++	+	0				0					0
From eye[1] in measles......	++	++	0											
From ear[1] in measles......	++	++	++	++	++	+	0		0					0
From throat in measles...	++	++	++	+	0				0					0
From throat in measles...	++	++	++	++	++	++	+	0	0					
From throat in measles...	++	++	++	++	++	++	++	++	0					0
From nose in measles......	++	++	++	0					0					0
From blood[1] in rubella....	0								++	++	0			0
From blood[1] in rubella....	0								++	++	++	++	0	0
From throat[1] in rubella...	0								++	++	++	++	0	0
From throat[1] in rubella*														
From throat in rubella....	0								+	+	+	±	0	
From throat in rubella....	++	0							0					0
From blood in rubella.....	++	0							0					0
From blood in rubella.....	++	0							0					0
Epidemic poliomyelitis....	0								+	+	0	0		0
Acute bronchitis..........	+	+	0						0					0

* Agglutinated spontaneously.

1. Strains used to immunize rabbits.

++ Marked clumping with complete clearing of the mixtures.

OPSONINS

The serum was diluted with normal salt solution, and then equal parts of washed human leukocytes and bacteria were added, making a total dilution of 1:15. At this dilution most of the normal opsonins were removed. The specimens were incubated at 37 C. for 15 minutes and the smears then stained with carbolthionin. Fifty polymorphonuclear leukocytes were counted and the number of cells taking part in phagocytosis noted. When the difference in phagocytic power between the immune and control serum was not marked, the experiments were repeated to be certain that the slight differences were of significance.

The serums were diluted to the point of opsonin extinction from time to time, the results corresponding then to the results obtained by diluting the

TABLE 2.

OPSONIN DETERMINATIONS

Sources of Cocci	Normal Rabbit Serum	Serum of Rabbit Immunized With Coccus from Measles	Serum of Rabbit Immunized With Coccus from Rubella
From measles: Blood	8	32	7
Blood	6	22	12
Eye	6	24	6
Ear	3	14	6
Nose	2	12	2
Throat	0	16	6
Throat	2	22	2
Throat	6	30	2
From rubella: Blood	4	8	30
Blood	0	2	26
Blood	7	9	36
Blood	2	2	16
Blood	0	2	7
Blood	4	6	28
Throat	8	8	32
Throat	7	11	22
Throat	6	6	42
Throat	0	2	30
Throat	0	2	14
Throat	3	2	13
Throat	0	0	20
Throat	4	2	28
Throat	0	0	9
Throat	3	3	13
Throat	2	2	7
Throat	6	7	16
Throat	4	2	10
Throat	4	8	16
Throat	6	8	8
Throat	1	0	4
Throat	2	8	20
Throat	5	7	16
Throat	0	1	9
Other sources:			
Arthritis	6	0	0
Endocarditis, blood	8	6	2
Tonsillitis	10	8	24
Tonsillitis	4	4	4
Tonsillitis	14	14	12
Tonsillitis	18	12	18
Tonsillitis	0	2	0
Acute chorea, throat	4	7	13
Acute chorea, throat	2	4	4
Pyorrhea	0	0	0
Pyorrhea	4	15	3
Spleen	8	6	8
Horse streptococcus	3	1	2
Poliomyelitis	5	4	7
Poliomyelitis	10	10	11
Poliomyelitis	3	3	1
Poliomyelitis	1	1	1
Poliomyelitis	0	1	0
Poliomyelitis	0	0	0
Pneumococcus, Type I	4	2	0
Pneumococcus, Type I	2	0	2
Pneumococcus, Type II	15	12	10
Pneumococcus, Type II	6	4	6
Pneumococcus, Type III	2	0	2
Pneumococcus, Type IV	10	6	8
Guinea-pig, blood	0	2	4
Streptococcus hemol.			
Tonsillitis	2	2	2
Scarlet fever, throat	6	10	9
Scarlet fever, blood	6	6	6
Scarlet fever, gland	20	20	20

serums to 1:15. The highest point of opsonin extinction by the measles serum was 1:960 for a heterologous strain. The highest point reached by the rubella strains was 1:4,800 for an homologous organism. The point of opsonin extinction for the control serum was 1:30.

The results (Table 2) show that the serum of an immune measles rabbit has marked opsonic power for measles diplococci, while the serum of an immune rubella rabbit and a control rabbit have about the same low opsonic content for these organisms. Further, that the serum of an immune rubella rabbit has increased opsonic power for rubella diplococci, while the measles and control rabbits have no such power.

The immune measles and rubella rabbit serum did not show any increase in opsonins for the 32 strains of diplococci and streptococci isolated from other sources, except in 3 instances, 1 a diplococcus from the throat of a nurse of a rubella patient, 1 from the throat of an acute chorea case, both of which corresponded morphologically to the rubella coccus and responded to the rubella rabbit serum, and small round diplococcus producing green colonies on blood agar isolated from a case of pyorrhea, reacted to the measles rabbit serum.

The opsonic determinations appear to show quite specific reactions which may be a help in differentiating the measles and rubella diplococci from each other and from similar organisms.

SUMMARY

Complement fixing bodies for the measles and rubella diplococci are present in the blood of measles and rubella patients to a slight extent as the symptoms subside.

The blood of rabbits injected with various strains of measles and rubella diplococci develop agglutinins and opsonins.

Agglutinins are demonstrable in the blood of immune measles rabbits often in high dilutions, both with homologous and heterologous measles strains. Satisfactory agglutination experiments are not possible as a rule until after long isolation of the diplococcus on account of spontaneous agglutination.

Agglutinins are present in the serum of immune rubella rabbits, but generally only for the homologous strains.

Specific opsonins are demonstrable in the blood of immune measles and rubella rabbits in high dilutions, and furnish the easiest and most reliable immunological method so far found, to differentiate measles and rubella diplococci from each other and from other nonhemolytic diplococci and streptococci.